N THE UNITED STATES PATENT AND TRADE MARK OFFICE

In re application of
Ichiro AZUMA *et al.*
Application No.: 09/743,750 Art Unit: 1645
Filed: January 16, 2001 Examiner: Vanessa L. Ford

For: FORMULATIONS USEFUL FOR IMMUNOTHERAPY FOR CANCERS CONTAINING BACTERIAL COMPONENT AS AN ACTIVE INGREDIENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Norimasa Koseki, a citizen of Japan and residing at Hyogo, Japan, say and declare as follows:

1. I received the degree of Ph.D from Hiroshima University in Japan in 1996.

2. I have been working at Sumitomo Pharmaceuticals (now Dainippon Sumitomo Pharma.) Technology Research & Development Center since 1993, studying pharmaceutical science and drug formulation.

3. I am a member of Injection and Topical Formulation Group in Formulation Laboratories.

4. I am an author or co-author of the following three papers related to cell biology and drug delivery to the CNS:

1) Cell Adhes. Commun. 1994 1: 355-366;
2) Cell Adhes. Commun. 1994 3: 463-474; and
3) Brain Res., 1999 849; 255-238.

5. Although I am not one of the inventors in U.S. Serial Number 09/743,750, I have been researching the subject matter of the said application since 1998 and am very familiar with the same.

6. I have conducted the following experiments related to the subject matter

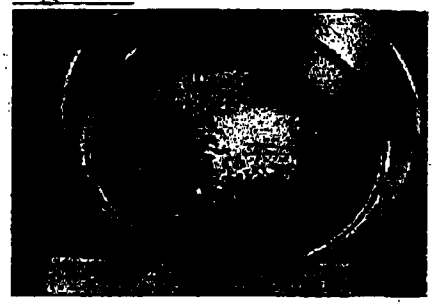
of the 09/743,750 application.

7. EXPERIMENTS

Experiment 1 (Comparison of oiled BCG-CWS)

A paste of BCG-CWS and an oil was prepared according to the method described in the present application, Yamamura *et al.*, and Zbar *et al.* BCG-CWS powder (25 mg) was measured and placed on a stainless steel dish (50 mm in diameter).

Figure 1



The BCG-CWS was dipped enough in oil in the case of the present invention (Sumitomo; 2a) as described in working Examples. But Yamamura formulation (2b) and Zbar formulation (2c) were partially oiled, and powdered BCG-CWS still co-existed in the case of Yamamura formulation (2b). The products or respective methods are shown in Fig. 2

Figure 2

a) Sumitomo (Squalane) b) Yamamura (Drakeol 6VR) c) Zbar(Drakeol 6VR)

These observations indicate that Yamamura formulation and Zbar formulation are not essentially suitable for preparing "emulsion" formation. It seems impossible to prepare well-dispersed emulsion solution using the oiled BCG-CWS described in Yamamura or Zbar.

Experiment 2 (Yamamura method)

(1) Compositions

Composition of the emulsion in Example 1 of Yamamura *et al.* (x 7)

Ingredients	contents
BCG-CWS	28 mg
Squalane	14 drops
Emulsifying solution containing:	total volume 7 mL
Mannitol	5.6%
Polysorbate 80	0.2 %

(2) Emulsification conditions

Device: Potter-Elvehjem type glass homogenizer

Procedures:

(3) Emulsifying solution (0.2%Tween 80/5.6% Mannitol)

Mannitol (28 g) was solved in distilled water (300 mL) and polysorbate 80 (1 g) was added. Distilled water was added to obtain 500 mL of emulsifying solution. Emulsifying solution (10 mL) was warmed to 60 °C for more than 30 min.

(4) Preparation of emulsion of Yamamura *et al.*

BCG-CWS (28 mg) was added in vessel of 10 mL Potter-Elvehjem type homogenizer and 14 drops of squalane were added by 1 mL syringe with 27G needle. The mixture was mixed at 1000 rpm for 5 minutes. Emulsifying solution (3 mL) which was warmed to 60°C was then added to the mixture and mixed at 1000 rpm for 5 min. Then, 4 mL of emulsifying solution was further added (7 mL in total) and mixed at 1000 rpm for 5 minutes..

Experiment 3 (Zbar method)

(1) Compositions

Composition of the emulsion in the Experiment of Zbar *et al.*

Ingredients	contents
BCG-CWS	25 mg
Drakeol	0.12 mL
Emulsifying solution containing:	total volume 16.7 mL
NaCl	0.85 %
Polysorbate 80	0.2 %

(2) Emulsification conditions

Device: Potter-Elvehjem type glass homogenizer

Procedures:

(3) Emulsifying solution (0.2%Tween 80/0.85% NaCl)

NaCl (0.85 g) was solved in distilled water (50 mL) and polysorbate 80 (0.2 g) was added. Distilled water was added to obtain 100 mL of emulsifying solution.

(4) Preparation of emulsion of Zbar *et al.*

BCG-CWS (25 mg) was added in vessel of 10 mL Potter-Elvehjem type homogenizer and 0.12 mL of Drakeol was added. BCG-CWS and Drakeol were mixed at 800 rpm enough to obtain smooth paste. Emulsifying solution (10 mL) was added to the mixture and mixed at 800 rpm for 5 minutes. The mixture was poured into a glass-tube, and the vessel of the homogenizer was washed with 6.7 mL of emulsifying solution and mixed at 800 rpm for 2-3 minutes. Emulsified mixture was combined together.

RESULTS

OBSERVATIONS (Visual examination)

(1) Observations of Yamamura's emulsion (Fig. 3)

Figure 3 (a): Aggregations in the bottom area

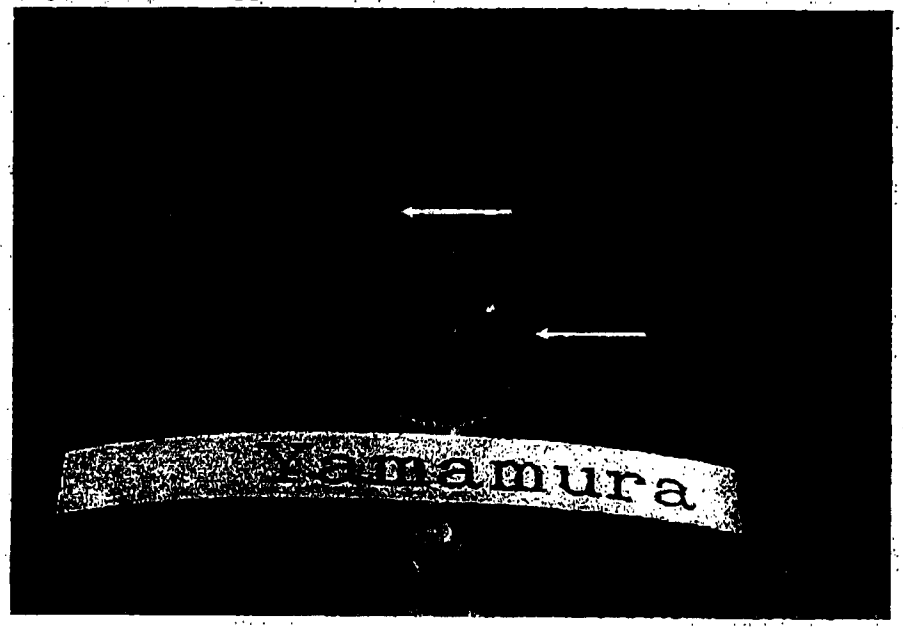


Figure 3 (b): Aggregations in the upper area

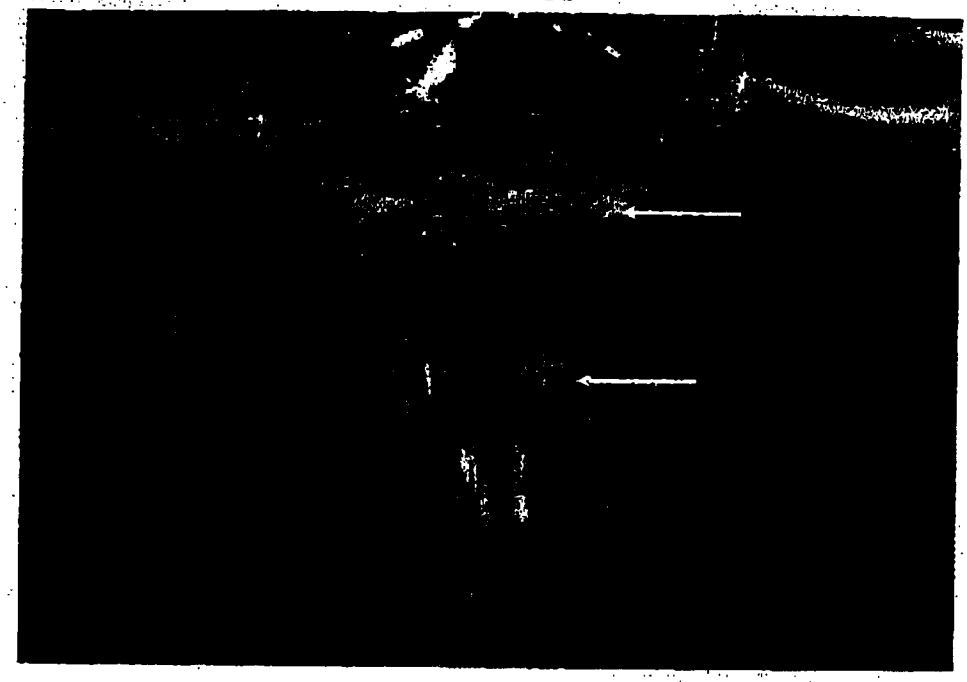


Figure 3 (a) shows that, in the emulsion prepared by Yamamura method, insoluble (not dispersed) BCG-CWS remained. Figure 3 (b) shows clearly that most of BCG-CWS remained in glass surface of the homogenizer as a white solid.

(2) Observations of Zbar's emulsion (Fig. 4)

Figure 4 (a): Aggregations in the bottom area

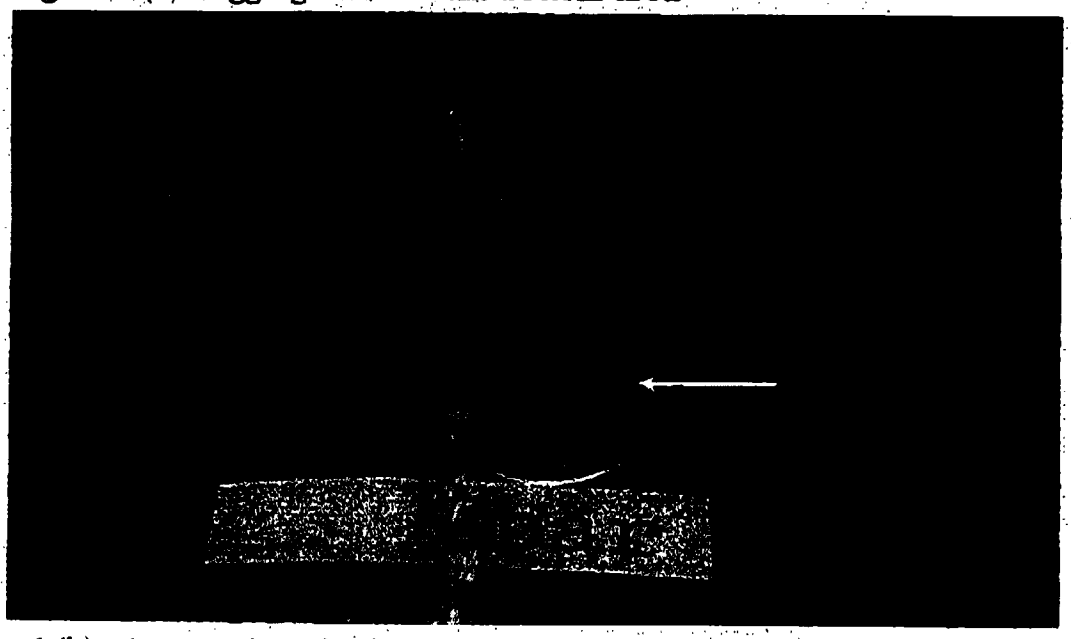
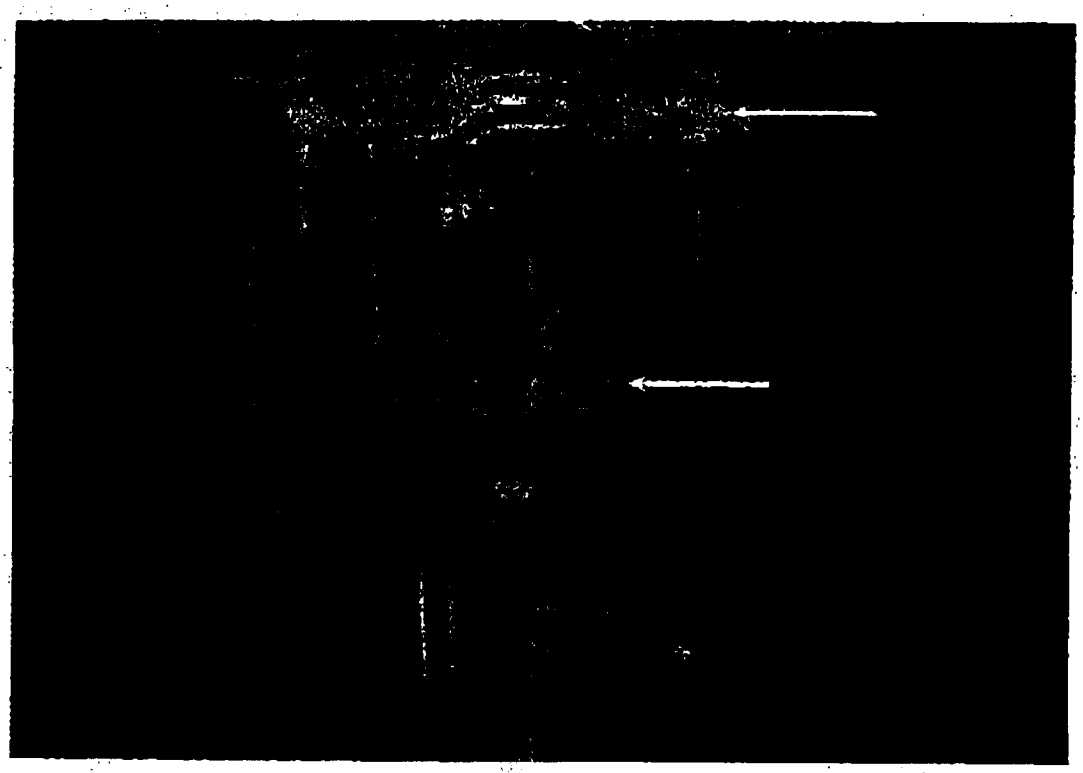


Figure 4 (b): Aggregations in the upper area



Figures 4 (a) and (b) show the same results in the case of Yamamura's emulsion.

As shown in the results of Experiment 2 or 3 above, we could not prepare an emulsion solution wherein BCG-CWS was well-dispersed by the method described in Yamamura *et al.* or Zbar *et al.*

(3) Emulsion of the present Invention (Fig. 5)

Figure 5: Overall picture of emulsion of the present Invention

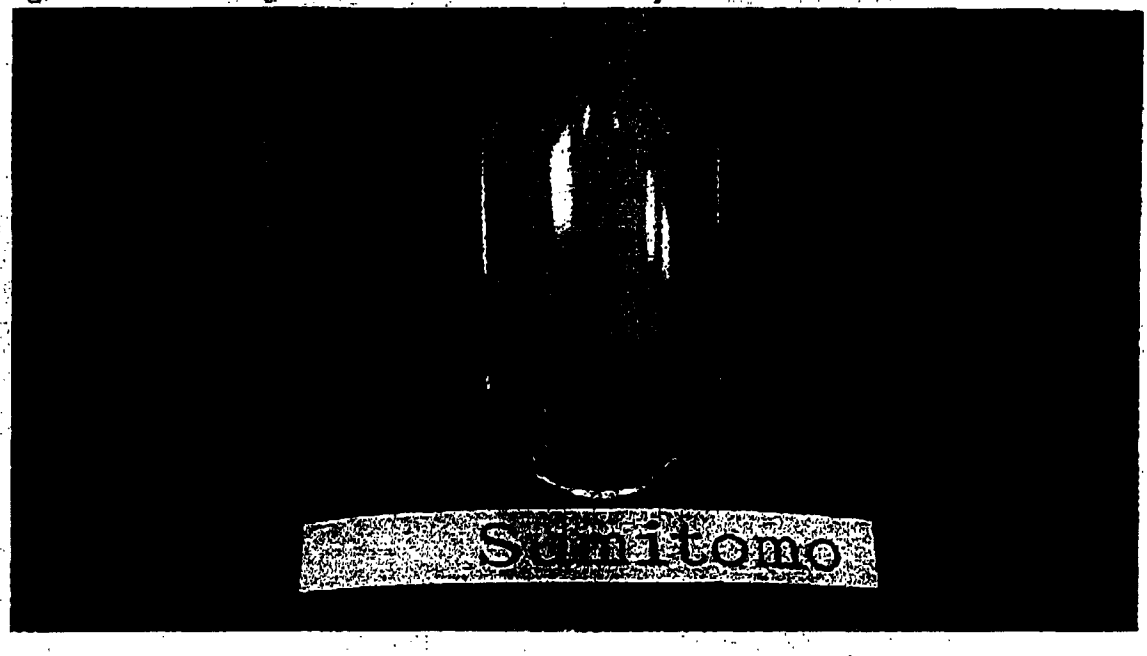


Figure 5 shows that, in the emulsion of the present invention prepared according to the manufacturing method described in the present application (4mg of BCG-CWS, 32mg of squalane, 1% of polysorbate 80 and 4.5% glycine are contained in 4ml of emulsion solution). The oiled BCG-CWS was well dispersed as emulsion in the solution. The BCG-CWS aggregation was not observed.

8. The undersigned declares further that all statement made herein of his own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that Such willful false statement may jeopardize the validity of above identified application or any patent issuing thereon.

Oct. 06, 2006
Date

N. Koseki
Dr. Norimasa Koseki